



# Thermo Scientific KingFisher Pure DNA Blood Kit

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**NOTE:** For more details on storing the kit reagents, refer to “[Storage Conditions](#)” on [page 6](#).



## Kit Content

Table 1-1. Thermo Scientific™ KingFisher™ Pure DNA Blood Kit

Item	KingFisher Pure DNA Blood Kit	
Cat. No.	98010196	98010496
Package size	96 samples	384 samples
Proteinase K	2 x 1 ml	8 x 1 ml
Lysis Buffer	15 ml	60 ml
KingFisher Magnetic Beads	2 x 1.4 ml	10.6 ml
Binding Buffer (conc.)*	23 ml	90 ml
Wash Buffer 1 (conc.)*	100 ml	4 x 100 ml
Wash Buffer 2 (conc.)*	40 ml	2 x 40 ml
Elution Buffer	30 ml	3 x 30 ml

\* Addition of ethanol required.

The KingFisher Pure Blood DNA Kit (Cat. No. 98010196 or 98010496) is intended for the purification of blood samples, using the Thermo Scientific™ KingFisher™ Flex with a 96 deep well head or the Thermo Scientific™ KingFisher™ Duo with a 12-pin head and a sample volume of 200 µl. The kit is also suitable for use with the KingFisher Flex with a 24 deep well head or the KingFisher Duo with a 6-pin head and a sample volume of 1 ml. When a 1 ml sample volume is used, the kit can handle approx. 25 or 100 samples, depending on the kit package size.

The user will need the KingFisher Flex or KingFisher Duo magnetic particle processor for conducting purification ([Table 1-2](#)). In addition, several common laboratory instruments and consumables are required to conduct

an efficient purification. For more details, refer to Chapter 5: “[Protocols and Pipetting Instructions](#)”. Suitable consumables for the KingFisher Duo and KingFisher Flex are listed in [Table 1-3](#) and [Table 1-4](#).

Storage Conditions

Upon arrival, store the Thermo Scientific™ KingFisher™ Magnetic Beads at +4°C. All the other buffers and reagents included in the KingFisher Pure DNA Blood Kit can be stored at room temperature (15–25°C). The Proteinase K solution is stable at room temperature until the seal of the vial is broken. After being opened, the vial should be stored at -20°C. The reagents are stable for up to three years from the manufacturing date.

Additional Reagents Required

- 96–100% ethanol (EtOH), molecular biology grade

Table 1-2. Thermo Scientific™ KingFisher™ magnetic particle processors

Cat. No.	Product
5400100	KingFisher Duo magnetic particle processor
5400630	KingFisher Flex magnetic particle processor with 96 deep well head
5400640	KingFisher Flex magnetic particle processor with 24 deep well head

Table 1-3. Thermo Scientific™ KingFisher™ Flex consumables

Cat. No.	Product	Package size
97002534	KingFisher Flex 96 tip comb for deep well magnet	100 pcs
97002610	KingFisher Flex 24 deep well tip comb and plate	50 pcs
97002540	KingFisher Flex 96 KF plate (200 µl)	48 pcs
95040450	Microtiter deep well 96 plate	50 pcs
95040460	Microtiter deep well 96 plate, sterile	50 pcs
95040470	KingFisher Flex 24 deep well plate	50 pcs
95040480	KingFisher Flex 24 deep well plate, sterile	50 pcs

Table 1-4. Thermo Scientific™ KingFisher™ Duo consumables

Cat. No.	Product	Package size
97003500	KingFisher Duo 12-tip comb for Microtiter deep well 96 plate	50 pcs
97003510	KingFisher Duo 6-tip comb for KingFisher Flex 24 deep well plate	48 pcs
95040450	Microtiter deep well 96 plate	50 pcs
95040460	Microtiter deep well 96 plate, sterile	50 pcs
97003520	KingFisher Duo elution strip	40 pcs
95040470	KingFisher Flex 24 deep well plate	50 pcs
95040480	KingFisher Flex 24 deep well plate, sterile	50 pcs
97003530	KingFisher Duo Combi pack for Microtiter deep well 96 plate  (tips combs, plates, and elution strips for 96 samples)	1 box





## Product Description

### Introduction

The KingFisher Pure DNA Blood Kit is designed for rapid automated purification of genomic DNA from whole blood samples using Thermo Scientific™ KingFisher™ instruments. The DNA purified using the KingFisher Pure DNA Blood Kit is of high quality and free of proteins, nucleases, and other contaminants or inhibitors. It is, therefore, suitable for direct use in many different downstream applications, such as PCR (polymerase chain reaction), restriction analysis, and several other enzymatic reactions.

### Intended Use

The KingFisher Pure DNA Blood Kit is developed for purification of DNA from whole blood samples using paramagnetic particles. The reagents and specific plastic consumables are designed for use with the KingFisher Flex and KingFisher Duo magnetic particle processors as part of an integrated system. The KingFisher Pure DNA Blood Kit enables extraction of DNA from fresh or frozen blood treated with EDTA (ethylenediaminetetraacetic acid) or citrate. The KingFisher Pure DNA Blood Kit is only intended for research use, not for clinical or diagnostic use. The user is responsible for validating the performance of the Thermo Scientific™ KingFisher™ instrument and the KingFisher Pure DNA Blood Kit for any particular use, as the performance of the kits has not been validated for any specific organism or downstream application.

### Principle and Procedure

The KingFisher Pure DNA Blood Kit uses magnetic-particle technology for DNA purification. The Thermo Scientific™ KingFisher™ technology combines the speed and efficiency of DNA purification with easy handling of magnetic

particles. The purification process requires no phenol/chloroform extraction and needs very little hands-on time.

The first step of the protocol lyses the blood cells, after which the DNA can bind to the surface of the KingFisher Magnetic Beads in the presence of the Binding Buffer. The KingFisher Magnetic Beads are highly reactive, superparamagnetic beads. The following three effective wash steps dispose of proteins, cell debris, and any residual contaminants, while the DNA bound to the KingFisher Magnetic Beads is transferred through the wash steps. Two different Wash Buffers are used, followed by an air drying step. High-quality DNA is eluted into the Elution Buffer and is ready for subsequent downstream processes.

## Kit Specifications

The KingFisher Pure DNA Blood Kit is designed for rapid automated preparation of highly pure DNA from whole blood using KingFisher magnetic particle processors. If a dispense step requiring the addition of the Binding Buffer is excluded, the approximate processing time is 30 minutes for the purification of 96 samples on the KingFisher Flex and 12 samples on the KingFisher Duo when the sample volume is 200  $\mu$ l. The obtained DNA can be used directly in various downstream applications.

Fresh or frozen blood treated with either EDTA or citrate can be used. The procedure is optimized for sample volumes of 200  $\mu$ l or 1 ml but the protocol can be scaled to sample volumes between these volumes. Typically 3–9  $\mu$ g of DNA can be purified from 200  $\mu$ l of whole blood with an  $A_{260}/A_{280}$  ratio of  $\geq 1.7$ –2.0. The DNA yields depend on the sample type, the sample storage, and the number of white blood cells.

## KingFisher Magnetic Particle Processors

The KingFisher magnetic particle processors are designed for the automated transfer and processing of magnetic particles in microplate format. The patented technology of the Thermo Scientific™ KingFisher™ systems is based on the use of magnetic rods covered with a disposable, specially designed tip comb and plates or tubes. Use only Thermo Scientific™ KingFisher™ plastic consumables, as use of products from other manufacturers may cause unsuitable mixing or even instability in the KingFisher instrument. The instrument functions without any dispensing or aspiration parts or devices. Samples and reagents, including magnetic particles, are dispensed onto the plates according to the corresponding instructions. Dispensing can be carried out manually or partially automatically using automatic

dispensers, for example, the Thermo Scientific™ Multidrop™ Combi and/or the Thermo Scientific™ Versette™. Thermo Scientific™ BindIt™ Software 3.2 can be used for running ready-made and optimized protocols for the Thermo Scientific™ KingFisher™ Pure Kits. It is also possible to transfer the developed protocol onto the onboard software and run it directly from the instrument. The KingFisher instruments provide a rapid and automated solution for complicated and time-consuming purification processes, resulting in high-purity DNA without risk of carryover or cross-contamination.

The KingFisher instrument family comprises four systems covering working volumes from 20 to 5000 µl. Each system consists of an instrument, specially designed plastic consumables, and the easy-to-use BindIt Software 3.2. The KingFisher Pure DNA Blood Kit is optimized and ready for use with the KingFisher Flex or KingFisher Duo.

KingFisher magnetic particle processors are intended for professional research use by trained personnel. Detailed information and user instructions for the KingFisher instruments can be found in their respective user manuals.

The BindIt Software 3.2 protocols optimized for the KingFisher Pure DNA Blood Kit are available for the KingFisher Flex 96 and 24 and KingFisher Duo. For more information, go to [www.thermoscientific.com/kingfisherinfo](http://www.thermoscientific.com/kingfisherinfo) or contact your local authorized distributor.

Table 2-1. Overview of KingFisher Flex and KingFisher Duo magnetic particle processors

	KingFisher Flex		KingFisher Duo	
	96 formats	24 format	12 format	6 format
Processing volume	20–1000 µl*	200–5000 µl	30–1000 µl*	200–5000 µl
Capacity	Up to 96 samples per run (sample volume 200 µl)	Up to 24 samples per run (sample volume 1 ml)	Up to 12 samples per run (sample volume 200 µl)	Up to 6 samples per run (sample volume 1 ml)
Magnetic head	96 inter-changeable formats for PCR plate, KingFisher Flex 96 KF plate, Microtiter deep well 96 plate	24 format for KingFisher Flex 24 deep well plate	12-pin magnet head for Microtiter deep well 96 plate	6-pin magnet head for KingFisher Flex 24 deep well plate
Plates	KingFisher Flex 96 KF plate (20–200 µl), 96 well PCR plate, skirted (20–100 µl), Microtiter deep well 96 plate (50–1000 µl)	KingFisher Flex 24 deep well plate (200–5000 µl)	Microtiter deep well 96 plate (50–1000 µl), KingFisher Duo elution strip (30–130 µl)	KingFisher Flex 24 deep well plate (200–5000 µl)
Tip combs	KingFisher Flex 96 tip comb for PCR magnets, KingFisher Flex tip comb for KF magnets, KingFisher Flex 96 tip comb for deep well magnets	KingFisher Flex 24 tip comb for deep well magnets	KingFisher Duo 12-tip comb	KingFisher Duo 6-tip comb
Heating temperature	Heating block temperature from +5°C above ambient room temperature to +115°C		Heating block temperature from +10°C to +75°C, elution strip +4°C to +75°C at room temperature	

\* See the details above on the Plates row.

## Safety Information

The following components of the KingFisher Pure DNA Blood Kit contain hazardous contents ([Table 3-1](#)).

Always wear a laboratory coat, disposable gloves and goggles, and follow the safety instructions provided in the kit instruction manual. It is recommended that Good Laboratory Practice (GLP) is followed to guarantee reliable analyses.

Table 3-1. Safety precautions

Reagent	Hazardous contents	Safety instructions
Lysis Buffer	Guanidium chloride, Polyethylene glycol octylphenol ether	Harmful if swallowed. Irritating to skin. Risk of serious damage to eyes. Harmful to aquatic organisms and may cause long-term adverse effects in the aquatic environment.  Do not breathe gas/fumes/vapor/spray. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. This material and its container must be disposed of as hazardous waste. Avoid release to the environment. Refer to special instruction/safety data sheets.

Continued

Cont.

Reagent	Hazardous contents	Safety instructions
Proteinase K	Proteinase, Tritirachium album serine	May cause sensitization by inhalation.  Do not breathe gas/fumes/vapor/spray. Wear suitable protective clothing. In case of accident or if you feel unwell, seek medical advice immediately. This material and its container must be disposed of as hazardous waste.
Binding Buffer (conc.)	Sodium perchlorate	Harmful if swallowed. Explosive when mixed with combustible material.  Keep away from combustible material. Do not breathe gas/fumes/vapor/spray. Take off immediately all contaminated clothing. Wear suitable protective clothing. This material and its container must be disposed of as hazardous waste.
Wash Buffer 1 (conc.)	Sodium perchlorate	Explosive when mixed with combustible material.  Keep away from combustible material. Take off immediately all contaminated clothing. This material and its container must be disposed of as hazardous waste.

# 4

## Storage Conditions and Preparation of the Reagents

### Storage Conditions

Upon arrival, store the KingFisher Magnetic Beads at +4°C. All the other buffers and reagents included in the KingFisher Pure DNA Blood Kit can be stored at room temperature (15–25°C). The Proteinase K solution is stable at room temperature until the seal of the vial is broken. After being opened, the vial should be stored at -20°C. The reagents are stable for up to three years from the manufacturing date.

### Preparation of the Binding Buffer and Wash Buffers

Add 96–100% ethanol to the bottles containing Binding Buffer, Wash Buffer 1, and Wash Buffer 2, as indicated below in [Table 4-1](#) prior to the first use.

Table 4-1. Instructions for the preparation of the buffers. Add the indicated volume per bottle.

	96 samples (Cat. No. 98010196)			384 samples (Cat. No. 98010496)		
	Binding Buffer	Wash Buffer 1	Wash Buffer 2	Binding Buffer	Wash Buffer 1	Wash Buffer 2
Concentrated buffer	23 ml	100 ml	40 ml	90 ml	100 ml	40 ml
Ethanol (96–100%)	23 ml	100 ml	200 ml	90 ml	100 ml	200 ml
Total volume	46 ml	200 ml	240 ml	180 ml	200 ml	240 ml

After preparing each solution, mark the bottle to indicate that the step has been completed. The buffers can be stored at room temperature.





## Protocols and Pipetting Instructions

Before beginning the DNA purification protocol, carefully read through the *Thermo Scientific™ KingFisher™ Flex User Manual* (Cat. No. N07669) or the *Thermo Scientific™ KingFisher™ Duo User Manual* (Cat. No. N12420), and the *Thermo Scientific™ BindIt™ Software for KingFisher Instruments version 3.2 User Manual* (Cat. No. N07974).

BindIt Software 3.2 protocols can be found at [www.thermoscientific.com/kingfisher](http://www.thermoscientific.com/kingfisher).

### Handling of KingFisher Magnetic Beads

A homogeneous distribution of the KingFisher Magnetic Beads in the container is essential before the beads are transferred to the wells in order to ensure a high consistency between the wells. To gain complete resuspension of the beads, shake the container vigorously or vortex briefly. The paramagnetic beads have a tendency to sediment relatively quickly.

### Instructions for KingFisher Flex with 96 Deep Well Plates

These instructions are intended for DNA purification from 200 µl of blood, using the KingFisher Pure DNA Blood Kit (Cat. No. 98010196 or 98010496) and the KingFisher Flex with 96 deep well plates.

When using the KingFisher Pure DNA Blood Kit for the first time, prepare the Binding Buffer, Wash Buffer 1, and Wash Buffer 2. For more instructions, refer to Chapter 4: [“Storage Conditions and Preparation of the Reagents”](#).

Check all the solutions in the kit for salt precipitation before each use. Redissolve precipitates by warming the solution at 37°C and equilibrate to room temperature (15–25°C).

- 1. Take four empty Thermo Scientific™ Microtiter™ deep well 96 plates and two empty Thermo Scientific™ KingFisher™ Flex 96 KF plates.
- 2. Prepare the **Sample plate** (i.e. a Microtiter deep well 96 plate).

Add the following reagents to the Sample plate and leave the plate at room temperature while the other plates are being filled.

Plate number	Plate type	Plate name	Content	Sample/reagent volume per well
1	Microtiter deep well 96 plate	Sample	Blood sample Lysis Buffer Proteinase K	200 µl 100 µl 20 µl

- 3. Fill the other **plates** as follows.

Plate number	Plate type	Plate name	Content	Reagent volume per well
2	Microtiter deep well 96 plate	Wash 1_1	Wash Buffer 1	900 µl
3		Wash 1_2	Wash Buffer 1	800 µl
4		Wash 2	Wash Buffer 2	800 µl
5	KingFisher Flex 96 KF plate	Elution	Elution Buffer	150 µl

- 4. Place a Thermo Scientific™ KingFisher™ Flex 96 tip comb for deep well magnets on a **Tip Plate** (i.e. an empty KingFisher Flex 96 KF plate).
- 5. Start the PURE\_DNABlood\_Flex96 protocol using the KingFisher Flex 96 and load the plates as instructed on the KingFisher Flex 96 instrument display.

Switch on the KingFisher Flex making sure that you are using the Thermo Scientific™ KingFisher™ Flex 96 deep well head and heating block.

Connect the PC with BindIt Software 3.2 to the KingFisher Flex. Start the PURE\_DNABlood\_Flex96 protocol. Insert the Tip Plate and the filled plates into the instrument as indicated on the KingFisher Flex display. After all the plates have been loaded into the instrument, the protocol will start.

When the KingFisher Flex is to be run as a standalone instrument, transfer the PURE\_DNABlood\_Flex96 protocol to the KingFisher Flex. The instructions for transferring the protocol can be found in Chapter 4: “Using the software” in the *BindIt Software for KingFisher Instruments version 3.2 User Manual*.

6. Add the KingFisher Magnetic Beads and Binding Buffer (supplemented with ethanol) to the Sample plate during the dispense step.

When the KingFisher Flex pauses at the dispense step after the lysis step at approximately 5 minutes after starting the protocol run, remove the plate from the instrument and add the well suspended magnetic bead suspension and Binding Buffer (supplemented with ethanol) to the **Sample plate**.

Plate number	Plate type	Plate name	Content	Reagent volume per well
1	Microtiter deep well 96 plate	Sample	KingFisher Magnetic Beads*	25 µl
			Binding Buffer	400 µl

\* Resuspend the KingFisher Magnetic Beads well by vortexing before use.

7. Place the Sample plate back into the instrument and press **Start**. After the pause, the protocol will continue to completion.
8. When the protocol is completed, remove the plates according to the instructions on the KingFisher Flex display and switch off the instrument. Store the purified DNA accordingly. The purified DNA is ready for use in downstream applications.

**NOTE:** The final DNA concentration in the Elution Buffer may increase if the purified DNA is eluted into a smaller than recommended volume of buffer, but this can slightly reduce the overall DNA yield.

## Instructions for KingFisher Flex with 24 Deep Well Plates

These instructions are intended for DNA purification from 1 ml of blood, using the KingFisher Pure DNA Blood Kit (Cat. No. 98010196 or 98010496) and the KingFisher Flex with 24 deep well plates.

When using the KingFisher Pure DNA Blood Kit for the first time, prepare the Binding Buffer, Wash Buffer 1, and Wash Buffer 2. For more instructions, refer to Chapter 4: [“Storage Conditions and Preparation of the Reagents”](#).

Check all the solutions in the kit for salt precipitation before each use. Redissolve precipitates by warming the solution at 37°C and equilibrate to room temperature (15–25°C).

- 1. Take six empty Thermo Scientific™ KingFisher™ Flex 24 deep well plates.
- 2. Prepare the **Sample plate**. Add the following reagents to the Sample plate, and leave the plate at room temperature while the other plates are being filled.

Plate number	Plate type	Plate name	Content	Sample/reagent volume per well
1	KingFisher Flex 24 deep well plate	Sample	Blood sample Lysis Buffer Proteinase K	1000 µl 500 µl 60 µl

- 3. Fill the other buffer **plates** as follows.

Plate number	Plate type	Plate name	Content	Reagent volume
2	KingFisher Flex 24 deep well plate	Wash 1_1	Wash Buffer 1	3700 µl
3		Wash 1_2	Wash Buffer 1	3700 µl
4		Wash 2	Wash Buffer 2	3700 µl
5		Elution	Elution Buffer	500 µl

- 4. Place a Thermo Scientific™ KingFisher™ Flex 24 tip comb on a **Tip Plate** (i.e. an empty KingFisher Flex 24 deep well plate).
- 5. Start the PURE\_DNABlood\_Flex24 protocol using the KingFisher Flex 24.

Switch on the KingFisher Flex making sure that you are using the Thermo Scientific™ KingFisher™ Flex 24 deep well head and heating block.

Connect the PC with BindIt Software 3.2 to the KingFisher Flex. Start the PURE\_DNABlood\_Flex24 protocol. Insert the Tip Plate and the filled plates into the instrument as indicated on the KingFisher Flex display. After all the plates have been loaded into the instrument, the protocol will start.

When the KingFisher Flex is to be run as a standalone instrument, transfer the PURE\_DNABlood\_Flex24 protocol to the KingFisher Flex. The instructions for transferring the protocol can be found in Chapter 4: “Using the software” in the *BindIt Software for KingFisher Instruments version 3.2 User Manual*.

6. Add the KingFisher Magnetic Beads and Binding Buffer to the Sample plate during the dispense step.

When the KingFisher Flex pauses at the dispense step after the lysis step at approximately 5 minutes after starting the protocol run, remove the plate from the instrument and add the well suspended magnetic bead suspension and Binding Buffer (supplemented with ethanol) to the **Sample plate**.

Plate number	Plate type	Plate name	Content	Reagent volume
1	KingFisher Flex 24 deep well plate	Sample	KingFisher Magnetic Beads* Binding Buffer	70 µl 1600 µl

\* Resuspend the KingFisher Magnetic Beads well by vortexing before use.

7. Place the Sample plates back into the instrument and press **Start**. After the pause, the protocol will continue to completion.
8. When the protocol is completed, remove the plates according to the instructions on the KingFisher Flex display and switch off the instrument. Store the purified DNA accordingly. The purified DNA is ready for use in downstream applications.

**NOTE:** The final DNA concentration in the Elution Buffer may increase if the purified DNA is eluted into a smaller than recommended volume of buffer, but this can slightly reduce the overall DNA yield.

## Instructions for KingFisher Duo with 12-pin Magnet Head and 96 Deep Well Plates

These instructions are intended for DNA purification from 200 µl of blood, using the KingFisher Pure DNA Blood Kit (Cat. No. 98010196 or 98010496) and the KingFisher Duo with 96 deep well plates.

When using the KingFisher Pure DNA Blood Kit for the first time, prepare the Binding Buffer, Wash Buffer 1, and Wash Buffer 2. For more instructions, refer to Chapter 4: “[Storage Conditions and Preparation of the Reagents](#)”.

- Check all the solutions in the kit for salt precipitation before each use. Redissolve precipitates by warming the solution at 37°C and equilibrate to room temperature (15–25°C).
1. Take one empty Microtiter deep well 96 plate and one Thermo Scientific™ KingFisher™ Duo elution strip.

2. Prepare the **Blood DNA plate** (i.e. a Microtiter deep well 96 plate).

Add the following reagents to the rows. Note that row B is reserved for the tip comb and should be left *empty*. Note that rows C, D, and E are also left *empty*.

Plate name and type	Row	Row name	Content	Sample/reagent volume per well
Blood DNA plate  Microtiter deep well 96 plate	A	Sample	Blood sample Lysis Buffer Proteinase K	200 µl 100 µl 20 µl
	B	Tip	12-tip comb	Empty
	C	Empty	Empty	Empty
	D	Empty	Empty	Empty
	E	Empty	Empty	Empty
	F	Wash 1_1	Wash Buffer 1	900 µl
	G	Wash 1_2	Wash Buffer 1	800 µl
	H	Wash 2	Wash Buffer 2	800 µl

3. Fill the KingFisher Duo elution strip as follows. Make sure that the elution strip is placed in the correct direction into the elution block. Ensure that the perforated end is facing towards the user and the Elution Buffer is pipetted into the correct wells.

Elution strip	Content	Reagent volume per well
KingFisher Duo elution strip	Elution Buffer	100 µl

4. Place a Thermo Scientific™ KingFisher™ Duo 12-tip comb into **row B** on a **Blood DNA plate**.
5. Start the PURE\_DNABlood\_Duo protocol using the KingFisher Duo and load the plate and elution strip.

Switch on the KingFisher Duo making sure that you are using the Thermo Scientific™ KingFisher™ Duo 12-pin magnet head and heating block.

Connect the PC with BindIt Software 3.2 to the KingFisher Duo. Start the PURE\_DNABlood\_Duo protocol. Insert the Blood DNA plate and elution strip into the instrument as indicated on the KingFisher Duo display and press **OK**. Make sure that the elution strip is placed in the correct direction into the elution block. Ensure that the perforated end is facing towards the user.

When the KingFisher Duo is to be run as a standalone instrument, transfer the PURE\_DNABlood\_Duo protocol to the KingFisher Duo. The instructions for transferring the protocol can be found in Chapter 4: “Using the software” in the *BindIt Software for KingFisher Instruments version 3.2 User Manual*.

6. Add the KingFisher Magnetic Beads and Binding Buffer (supplemented with ethanol) to row A during the dispense step.

When the KingFisher Duo pauses at the dispense step after the lysis step at approximately 5 minutes after starting the protocol run, remove the plate from the instrument and add the well suspended magnetic bead suspension and Binding Buffer (supplemented with ethanol) to **row A** on the **Blood DNA plate**.

Row	Row name	Content	Reagent volume per well
A	Sample	KingFisher Magnetic Beads*	25 µl
		Binding Buffer	400 µl

\* Resuspend the KingFisher Magnetic Beads well by vortexing before use.

7. Place the Blood DNA plate back into the instrument and press **OK**. After the pause, the protocol will continue to completion.
8. When the protocol is completed, remove the plate and elution strip according to the instructions on the KingFisher Duo display and switch off the instrument. Store the purified DNA accordingly. The purified DNA is ready for use in downstream applications.

**NOTE:** The final DNA concentration in the Elution Buffer may increase if the purified DNA is eluted into a smaller than recommended volume of buffer, but this can slightly reduce the overall DNA yield.

## Instructions for KingFisher Duo with 6-pin Magnet Head and 24 Deep Well Plates

These instructions are intended for DNA purification from 1 ml of blood, using the KingFisher Pure DNA Blood Kit (Cat. No. 98010196 or 98010496) and the KingFisher Duo with 6-pin magnet head and 24 deep well plates.

When using the KingFisher Pure DNA Blood Kit for the first time, prepare the Binding Buffer, Wash Buffer 1, and Wash Buffer 2. For more instructions, refer to Chapter 4: [“Storage Conditions and Preparation of the Reagents”](#).

Check all the solutions in the kit for salt precipitation before each use. Redissolve precipitates by warming the solution at 37°C and equilibrate to room temperature (15–25°C).

1. Take two empty Thermo Scientific™ KingFisher™ Flex 24 deep well plates.
2. Prepare the **Blood DNA plates 1 and 2**. Add the following reagents to two KingFisher Flex 24 deep well plates. Note that row B on the Blood DNA plate 1 is reserved for the tip comb and should be left *empty*. Note that row C on the Blood DNA plate 1 and row B on the Blood DNA plate 2 are also left *empty*.

Plate name and type	Row	Row name	Content	Sample/reagent volume
Blood DNA plate 1  KingFisher Flex 24 deep well plate	A	Sample	Blood sample Lysis Buffer Proteinase K	1000 µl 500 µl 60 µl
	B	Tip	Empty	Empty
	C	Empty	Empty	Empty
	D	Wash 1_1	Wash Buffer 1	3700 µl

Plate name and type	Row	Row name	Content	Sample/reagent volume
Blood DNA plate 2  KingFisher Flex 24 deep well plate	A	Elution	Elution Buffer	500 µl
	B	Empty	Empty	Empty
	C	Wash 2	Wash Buffer 2	3700 µl
	D	Wash 1_2	Wash Buffer 1	3700 µl



3. Place a Thermo Scientific™ KingFisher™ Duo 6-tip comb into **row B** on the **Blood DNA plate 1**.
4. Start the PURE\_DNABlood\_Duo1ml protocol using the KingFisher Duo and load the plates.

Switch on the KingFisher Duo making sure that you are using the Thermo Scientific™ KingFisher™ Duo 6-pin magnet head and heating block.

Connect the PC with BindIt Software 3.2 to the KingFisher Duo. Start the PURE\_DNABlood\_Duo1ml protocol. Insert the Blood DNA plates into the instrument as indicated on the KingFisher Duo display and press **OK**. After all the plates have been loaded into the instrument, the protocol will start.

When the KingFisher Duo is to be run as a standalone instrument, transfer the PURE\_DNABlood\_Duo1ml protocol to the KingFisher Duo. The instructions for transferring the protocol can be found in Chapter 4: "Using the software" in the *BindIt Software for KingFisher Instruments version 3.2 User Manual*.

6. Add the KingFisher Magnetic Beads and Binding Buffer to row A on the Blood DNA plate 1 during the dispense step.

When the KingFisher Duo pauses at the dispense step after the lysis step at approximately 5 minutes after starting the protocol run, remove the Blood DNA plate 1 from the instrument and add the well suspended magnetic bead suspension and Binding Buffer (supplemented with ethanol) to **row A** on the **Blood DNA plate 1**.

Plate name and type	Row	Row name	Content	Reagent volume
Blood DNA plate 1	A	Sample	KingFisher Magnetic Beads*	70 µl
KingFisher Flex 24 deep well plate			Binding Buffer	1600 µl

\* Resuspend the KingFisher Magnetic Beads well by vortexing before use.

7. Place the Blood DNA plate 1 back into the instrument and press **OK**. After the pause, the protocol will continue to completion.

8. When the protocol is completed, remove the plates according to the instructions on the KingFisher Duo display and switch off the instrument. Store the purified DNA accordingly. The purified DNA is ready for use in downstream applications.

**NOTE:** The final DNA concentration in the Elution Buffer may increase if the purified DNA is eluted into a smaller than recommended volume of buffer, but this can slightly reduce the overall DNA yield.

## Quantification and Determination of the Purity of DNA

It is recommended to measure the absorbance at 320 nm, 280 nm, and 260 nm. The concentration of DNA can be defined with the absorbance at 260 nm ( $A_{260}$ ). One unit at 260 nm corresponds to 50 µg of DNA per ml. The ratio between the  $A_{260}/A_{280}$  indicates the purity of the DNA. The value for DNA should be  $\geq 1.7$ –2.0.

It is recommended that  $A_{320}$  correction is used for the absorbance values. Subtract the  $A_{320}$  from the  $A_{260}$  and  $A_{280}$  ratios to remove the effects of carryover of the magnetic particles.

- Concentration of DNA sample =  $50 \mu\text{g/ml} \times (A_{260} - A_{320}) \times \text{dilution factor}$
- Total amount of DNA isolated = concentration x volume of sample in ml
- Purity of DNA sample =  $(A_{260} - A_{320}) / (A_{280} - A_{320})$

## General Information

### Reagent Specificity and Volumes

A reagent must not be used with any kit other than that for which it is intended. It is strongly recommended that the volume of reagents in each well is kept within the limits specified in the *KingFisher Flex User Manual* or the *KingFisher Duo User Manual* to avoid spillover and to maximize efficiency of performance.

### Handling of Magnetic Beads

The KingFisher Magnetic Beads should be mixed thoroughly before use to avoid the risk of transferring variable amounts of the beads to the respective wells. The amount of beads in the wells affects the yield of the purified DNA.

### Binding, Wash, and Elution Steps

The binding between the purified DNA and the KingFisher Magnetic Beads is strong in the presence of a chaotropic salt. The binding will remain throughout the wash steps until the elution where the DNA is released.

The volume of the Elution Buffer can be modified depending on user requirements concerning the purified DNA concentration. The final DNA concentration in the Elution Buffer may increase if the purified DNA is eluted into a smaller than recommended volume of the buffer, but this can slightly reduce the overall DNA yield. The modifications of the elution step must be done in BindIt Software 3.2 and according to the volume ranges suitable for the KingFisher instrument. The table below indicates the available elution volumes of the KingFisher instruments.

Table 6-1. Available elution volumes of the KingFisher Flex and KingFisher Duo

KingFisher instrument	Elution volumes
KingFisher Flex with 96 deep well head, elution in a KingFisher Flex 96 KF plate	50–150 µl
KingFisher Flex with 96 deep well head, elution in a Microtiter deep well 96 plate	50–1000 µl
KingFisher Flex with 24 deep well head	200–5000 µl
KingFisher Duo with 12-pin magnet head, elution in an elution strip	30–130 µl
KingFisher Duo with 12-pin magnet head, elution in a Microtiter deep well 96 plate	50–1000 µl

To maximize the yield of purified DNA, avoid the lowest permitted volumes of Elution Buffer in the KingFisher instruments. The Elution Buffer should cover the KingFisher Magnetic Beads completely, and any possible sedimented magnetic beads should be completely resuspended. In addition, the volume of the Elution Buffer should be adequate for efficient mixing of the beads in order to obtain a maximal release of the purified DNA from the beads.

## Decontamination and Disinfection of Sample Material

You should decontaminate the sample material and the reagents and plastics that have been in contact with the sample material in order to minimize the risk of contamination. Use a decontaminant, such as Virkon™, paying due attention to the manufacturer's instructions. You should also take care of the appropriate treatment and/or disposal of waste.



## Troubleshooting

Problem	Possible cause and actions
Low DNA yield	<p>Make sure that ethanol was added to the Binding Buffer, Wash Buffer 1, and Wash Buffer 2 before use. Follow the instructions for "<a href="#">Preparation of the Binding Buffer and Wash Buffers</a>" on <a href="#">page 15</a>.</p> <p>Do not let the KingFisher Magnetic Beads dry as this may result in lower elution efficiency.</p> <p>Efficient lysis of the blood cells increases the DNA yield.</p> <p>Prolonged storage or repeated freeze/thaw cycles of the sample material may reduce the DNA yield.</p> <p>There should be an adequate volume of the Elution Buffer to cover the KingFisher Magnetic Beads completely during the elution step.</p> <p>Use only Thermo Scientific plates, strips, and tip combs with the KingFisher instruments. Use of products from other manufacturers may cause unsuitable mixing and affect the yield of purified DNA.</p>
Low purity	<p>Insufficient washing causes impurities in the Elution Buffer.</p> <p>Blood clots in the sample cause impurities in the purified DNA.</p>

Continued

Cont.

Problem	Possible cause and actions
Magnetic particles remaining in the sample or elution well	<p>Starting material that is too viscose prevents efficient collection of the KingFisher Magnetic Beads from the lysed sample. The magnetic rods will not be able to collect all the particles unless the viscose samples are diluted before the beginning of the purification. Improper lysis may also cause problems collecting the KingFisher Magnetic Beads.</p> <p>If the KingFisher Magnetic Beads are inefficiently collected from the Elution Buffer, the addition of a small amount of detergent (e.g. Tween™ 20) may improve the results. Alternatively, centrifuge the eluates or place them on a magnet for a few minutes to collect the residual beads at the bottom of the well. Carryover of the KingFisher Magnetic Beads does not affect most downstream processes.</p> <p>KingFisher Magnetic Beads that occasionally remain attached to the tip combs at the end of the process do not affect the DNA yield, as the DNA has already been released from the KingFisher Magnetic Beads into the Elution Buffer.</p>
Magnetic particles remaining in the sample or elution well	If the KingFisher magnetic particle processor does not work properly, refer to the relevant user manual of the KingFisher instrument in use.
Inhibition of downstream enzymatic reactions	If the purified DNA contains residual salt, use the correct order for the Wash Buffers. Always wash the KingFisher Magnetic Beads with Wash Buffer 1 first and then proceed with Wash Buffer 2.

# B

## Ordering Information

Table B-1. KingFisher Pure DNA Blood Kits

Cat. No.	Product	Package size
98010196	KingFisher Pure DNA Blood Kit	96
98010496	KingFisher Pure DNA Blood Kit	384

Table B-2. KingFisher Flex consumables

Cat. No.	Product	Package size
97002514	KingFisher Flex 96 tip comb for PCR magnet	80 pcs
97002524	KingFisher Flex 96 tip comb for KF magnet	100 pcs
97002534	KingFisher Flex 96 tip comb for deep well magnet	100 pcs
97002610	KingFisher Flex 24 deep well tip comb and plate	50 pcs
97002540	KingFisher Flex 96 KF plate (200 µl)	48 pcs
95040450	Microtiter deep well 96 plate	50 pcs
95040460	Microtiter deep well 96 plate, sterile	50 pcs
95040470	KingFisher Flex 24 deep well plate	50 pcs
95040480	KingFisher Flex 24 deep well plate, sterile	50 pcs

Table B-3. KingFisher Duo consumables

Cat. No.	Product	Package size
97003500	KingFisher Duo 12-tip comb for Microtiter deep well 96 plate	50 pcs
97003510	KingFisher Duo 6-tip comb for KingFisher Flex 24 deep well plate	48 pcs
95040450	Microtiter deep well 96 plate	50 pcs
95040460	Microtiter deep well 96 plate, sterile	50 pcs
97003520	KingFisher Duo elution strip	40 pcs
95040470	KingFisher Flex 24 deep well plate	50 pcs
95040480	KingFisher Flex 24 deep well plate, sterile	50 pcs
97003530	KingFisher Duo Combi pack for Microtiter deep well 96 plate (tips combs, plates, and elution strips for 96 samples)	1 box



## Notes

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**Thermo Fisher Scientific**  
81 Wyman Street  
Waltham, MA 02451

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